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ACTIVITY PATTERNS AND OSMOSENSITIVITY OF RAT SUPRAOPTIC NEURONES IN PERFUSED HYPOTHALAMIC EXPLANTS

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SUMMARY

1. Extracellular recordings were obtained *in vitro* from supraoptic neurones in an explant of rat hypothalamus maintained viable through intravascular perfusion with artificial media.

2. The spontaneous activity observed from 64% of cells included continuously active neurones (mean frequency $3{\cdot}2 \pm 0{\cdot}8$ Hz) located throughout the nucleus, and phasically active neurones ($8{\cdot}6 \pm 0{\cdot}6$ Hz) located predominantly in the caudal half of the nucleus.

3. Supraoptic neurones displayed antidromic activation (latency range 6·5–18·2 ms) following stimulation of the pituitary stalk and orthodromic excitatory or inhibitory responses following stimulation in the anteroventral third ventricular (AV3V) region. Synaptic responses were reversibly abolished during perfusion with media containing 12 mM-Mg^{2+}.

4. A 15–40 mosmol/l increase in the osmotic pressure of the perfusion media, through addition of NaCl, sucrose or mannitol, prompted nineteen of twenty-three cells to increase their discharge frequency. The patterns of response included the induction or simple increase in continuous firing frequency, the induction or enhancement of phasic or bursting activity and a change from continuous to phasic activity.

5. Similar responses to an osmotic stimulus were obtained from thirteen of twenty-one supraoptic neurones, including two phasic neurones, where synaptic activity had been abolished during perfusion with 12 mM-Mg^{2+}.

6. Osmosensitivity appeared to be selective for supraoptic cells; no significant change in firing frequency was observed from any of six cells recorded in the lateral hypothalamus or thirteen cells recorded in the medial hypothalamus during exposure to a change in osmotic pressure of +20 to +40 mosmol/l, using either control media (seven cells) or media containing 12 mM-Mg^{2+} (twelve cells).

7. These observations indicate that supraoptic neurones maintained *in vitro* can display spontaneous, antidromic, orthodromic and osmotically induced activity patterns identical to those observed with *in vivo* recordings. The persistence of a reduced osmosensitivity among supraoptic neurones in the absence of synaptic transmission indicates that although these cells can function as osmoreceptors, their

osmosensitivity may be enhanced through synaptic input from adjacent neurones, possibly located in the AV3V area.

8. The presence of phasic activity among supraoptic neurones maintained in media where synaptic transmission has been abolished suggests that the mechanisms responsible for such activity patterns are endogenous membrane properties of a subpopulation of supraoptic neurones.

INTRODUCTION

An increase in plasma osmolarity is a potent physiological stimulus to enhance release of the neurohypophyseal hormones vasopressin and oxytocin (Dunn, Brennan, Nelson & Robertson, 1973; Brimble, Dyball & Forsling, 1978). This neuroendocrine 'reflex' may involve the participation of peripheral osmoreceptors, e.g. in the hepatic portal vein area (Baertschi & Vallet, 1981) and centrally located osmosensitive neurones (cf. Bie, 1980 for review). As originally proposed by Jewell & Verney (1957), the site for translation of the osmotic stimulus into an effective hormonal response ultimately focuses on the area of the supraoptic nucleus. Some of the synaptic pathways whereby information on plasma or cerebrospinal fluid osmolality may be relayed to supraoptic neurones have been recently described (Swanson & Sawchenko, 1983), as have chemical factors and neurotransmitters that influence the osmotic response (Hayward, 1977; Sladek & Knigge, 1977; Sladek & Joynt, 1979, 1980). Nevertheless, controversy still exists as to whether the supraoptic neurosecretory neurones are osmoreceptors. Their failure to respond consistently to an osmotic stimulus during recordings *in vitro* in hypothalamic slices (Haller & Wakerley, 1980) or *in vivo* following hypothalamic deafferentation (Dyball & Prilusky, 1981) suggests that supraoptic neurosecretory cells may not be osmosensitive, or that transduction of the osmotic response into hormone release requires intact synaptic connexions. On the other hand, there is a striking similarity in the manner of the *in vivo* response of supraoptic neurosecretory neurones to systemic increases in plasma osmotic pressure (cf. Brimble & Dyball, 1977) and their response to local applications of hypertonic NaCl (Leng, 1980). Furthermore, intracellular recordings in hypothalamic slices have demonstrated that supraoptic neurones do respond to an osmotic stimulus even when synaptic transmission is abolished (Mason, 1980; Abe & Ogata, 1982). These latter observations support the proposal that the supraoptic neurone can function as an osmoreceptor.

In vitro electrophysiological studies can provide valuable information on the behaviour of neurosecretory neurones and the involvement of synaptic mechanisms in an integrated response to osmotic stimuli. We have recently reported on the suitability of an explant of rat hypothalamus maintained through intravascular perfusion for such *in vitro* experimentation (Bourque & Renaud, 1983*a*). We now report that extracellular activity patterns of supraoptic neurones recorded *in vitro* and subjected to 'physiological' increases in the osmotic pressure of the perfusion media closely resemble *in vivo* observations, even when synaptic transmission is abolished in the presence of 12 mM-Mg^{2+}. These observations have been reported briefly (Bourque & Renaud, 1982).

METHODS

Basal diencephalic explants were prepared from ether-anaesthetized male Sprague–Dawley rats as described in Bourque & Renaud (1983*b*). Each explant was placed in a heated chamber (33–34 °C), fixed to a Sylgard slab with insect pins and maintained viable through gravity-fed perfusion (1·0–1·8 ml/min) of oxygenated (95 % O_2–5 % CO_2) artificial media delivered via a 0·15–0·20 mm pipette inserted into the right anterior cerebral artery through the stump of the carotid artery. The media (pH 7·35–7·45) contained (mM): NaCl, 126; KCl, 3·0; KH_2PO_4, 1·25; $MgSO_4$, 1·30; $CaCl_2$, 1·2–2·4; $NaHCO_3$, 25·9; glucose, 10·0.

A bipolar insulated nichrome electrode inserted transaxially into the main substance of the pituitary stalk was utilized to activate axons of supraoptic neurones. Another bipolar concentric electrode was positioned behind the anterior communicating artery in the anteroventral third ventricular area (AV3V) and served to activate some of the afferent pathways to supraoptic neurones from this area. These electrodes were connected to isolated stimulation units that delivered 0·05 ms current pulses (0·2–0·5 mA) controlled by a programmable clock.

Extracellular recordings were obtained through 2 M-NaCl-filled glass micropipettes with impedances of 5–15 MΩ. Signals were amplified conventionally, and led through a spike processor (Medical Systems Corp.) to a PDP 11/40 computer programmed for spike train analysis. All recordings were restricted to the supraoptic nucleus proper, located within 250 μm of the lateral edge of the optic tract and less than 400 μm from the ventral brain surface. Confirmation of this recording site was ascertained in separate experiments where cells were marked intracellularly with the fluorescent dye Lucifer Yellow and identified in 300 μm sections through the nucleus.

For the purpose of testing cells in the absence of synaptic influences, control media were replaced by media containing 12 mM-$MgSO_4$ isotonically replaced by NaCl (cf. Fig. 1). In order to test for osmosensitivity, the perfusion media (≈ 280–285 mosmol/l determined by freezing point osmometry) were replaced by solutions with a 15–40 mosmol/l increase in osmotic pressure achieved by the addition of either NaCl, sucrose or mannitol, and maintained for a minimum of 8 min (maximum of 30 min) before returning to control media.

Since no changes in excitability were detected from any of seven supraoptic neurones tested with media containing a + 10 mosmol/l change in osmotic pressure, an increase of 15 mosmol/l was taken to represent the threshold for induction of enhanced action potential generation. Neurones were arbitrarily tested with media containing high osmotic pressures (+ 40 mosmol/l) in order to provoke maximum short term responses to an osmotic stress.

RESULTS

Spontaneous activity

Extracellular recordings obtained from 64 % of supraoptic neurones in the unstimulated preparation displayed spontaneous discharge patterns that closely resembled those observed during recordings *in vivo* (cf. Poulain & Wakerley, 1982), i.e. continuous irregular activity and phasic or bursting activity. Continuously active cells, found throughout the nucleus, had firing frequencies that ranged betwen 0·1 and 15 Hz (mean 3·2 ± 0·8 Hz, S.E. of the mean; $n = 20$). The majority (86 %) of phasically active cells ($n = 22$) were encountered in the caudal half of the nucleus, posterior to the bifurcation of the anterior and middle cerebral artery. During active periods, these neurones displayed firing frequencies between 3 and 25 Hz (mean 8·6 ± 0·6 Hz) with burst lengths ranging from 4 to 210 s (mean 20·9 ± 0·8 s), and silent periods ranging between 3 and 90 s (mean 34·3 ± 1·7 s).

Antidromic activation

In preparations where it was obvious that minimal damage had been inflicted to the pituitary stalk during the removal of the brain from the cranial cavity, and where

an intact vascular network insured good perfusion of the deeper portion of the medial hypothalamus, antidromic activation of as many as 40 % of supraoptic neurones could be observed following pituitary stalk stimulation. Antidromic latencies ranged between 6·5 and 18·2 ms (mean 11·3 ± 0·8 ms; $n = 32$) and included samples from silent, continuous and phasic neurones. On the premise that nearly all supraoptic nucleus neurones project their axons into the neurohypophysis (Sherlock, Field & Raisman, 1975), failures to evoke antidromic activation from supraoptic neurones were interpreted as arising from some traumatic or anoxic damage to their axons.

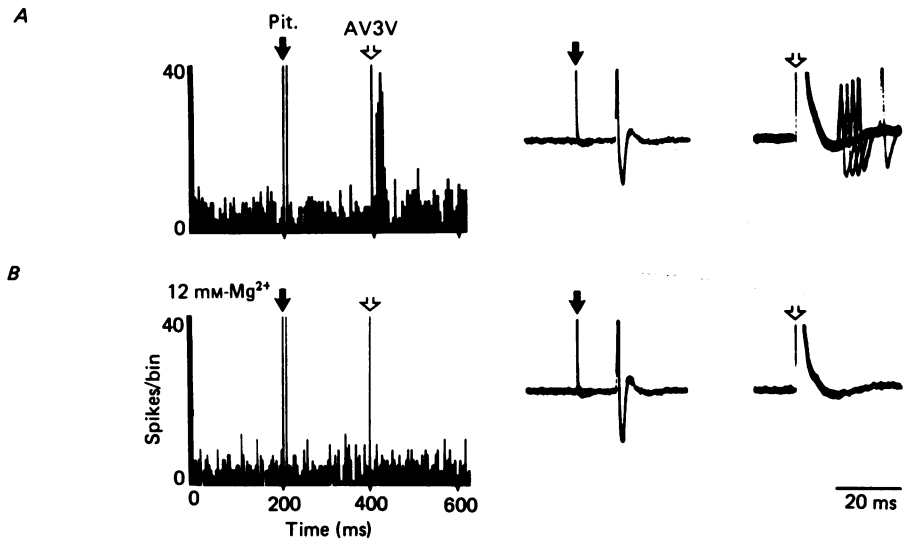

Fig. 1. Perfusion media containing 12 mM-Mg^{2+} blocks synaptic but not antidromic activation. *A*, post-stimulus histograms (resolution of 4 ms/bin) and superimposed oscilloscope traces obtained from a supraoptic neurosecretory neurone illustrate evoked action potentials at a constant latency following stimulation in the posterior pituitary (Pit., filled arrows) and at variable latencies following AV3V stimulation (open arrows). *B*, failure of AV3V evoked responses, but not the antidromic responses, during perfusion with media containing 12 mM-Mg^{2+}.

Synaptic events

The majority (93 %) of supraoptic neurones displayed orthodromic response patterns following stimulation in the AV3V region. For any given location of the AV3V stimulating electrode, only one type of synaptic event (i.e. excitatory or inhibitory) could usually be elicited from most neurones in that preparation, suggesting that functionally different pathways to the supraoptic nucleus arise within or course through the AV3V area. Orthodromic activation, recorded from forty-six neurones, was characterized by the appearance of single action potentials at variable latencies ranging between 4 and 15 ms (Fig. 1). Orthodromic inhibition, noted in ten cells, displayed latencies of 10–17 ms and lasted up to 120 ms (cf. Fig. 5 in Bourque & Renaud, 1983*b*). All synaptically mediated events were abolished 6–8 min following the onset of perfusion with media containing 12 mM-Mg^{2+} (Fig. 1).

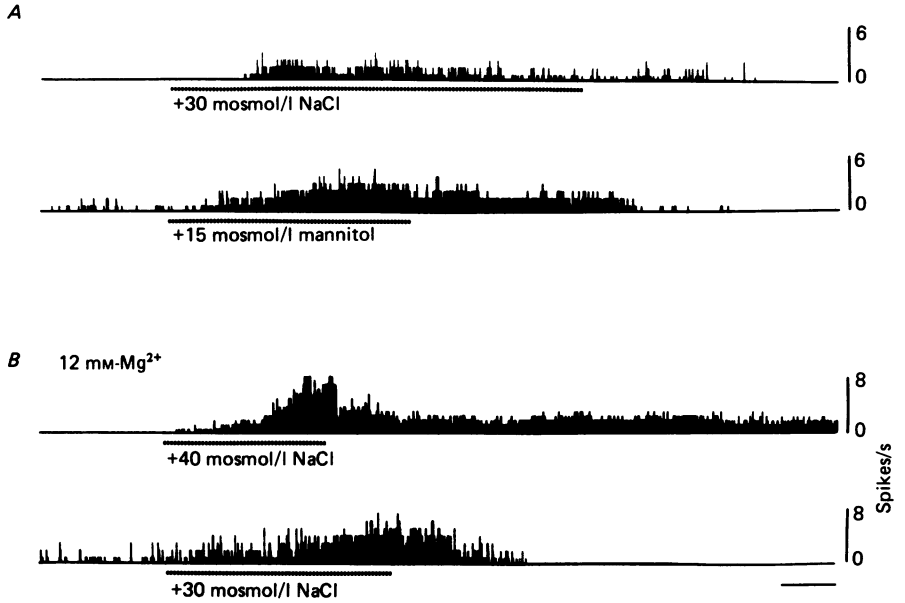

Fig. 2. *A*, rate-meter records from different supraoptic neurones illustrate the induction (upper trace) or enhancement (lower trace) of continuous firing by an increase in the osmotic pressure of the perfusion media during the period indicated by the dotted line. *B*, osmotic responses obtained from two other supraoptic neurones, this time in media containing 12 mM-Mg^{2+}.

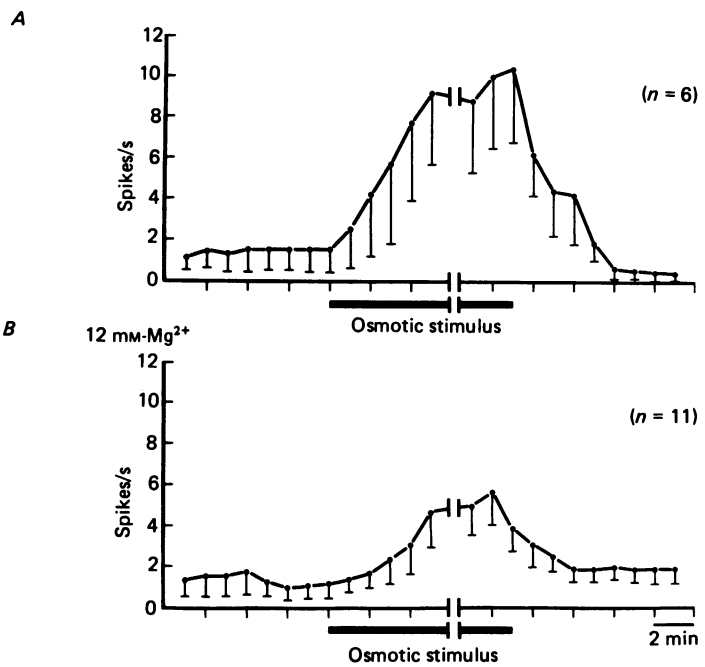

Fig. 3. *A*, plot of the mean (±S.E. of the mean) firing frequency of six supraoptic neurones that displayed continuous activity, illustrating the increase in firing during perfusion with hyperosmotic media (+20 to +40 mosmol/l using Na^+, sucrose or mannitol). *B*, data obtained from eleven other continuously active neurones exposed to similar osmotic stimuli but recorded in media containing 12 mM-Mg^{2+}. Diagrams are normalized to account for variations in the length of cell exposure to the hyperosmotic media, indicated by the filled horizontal bar.

Response to osmotic stimuli

A 15–40 mosmol/l increase in the osmotic pressure of the standard perfusion media was followed by an increase in action potential frequency for nineteen of twenty-three neurones tested. Effective osmotic mediators included sucrose (five of six cells), mannitol (six of seven cells), or NaCl (eight of ten cells). Latencies varied between 0·5 and 8·0 min, while the induced changes in excitability usually persisted for several minutes following a return to control media. Several patterns of response were observed.

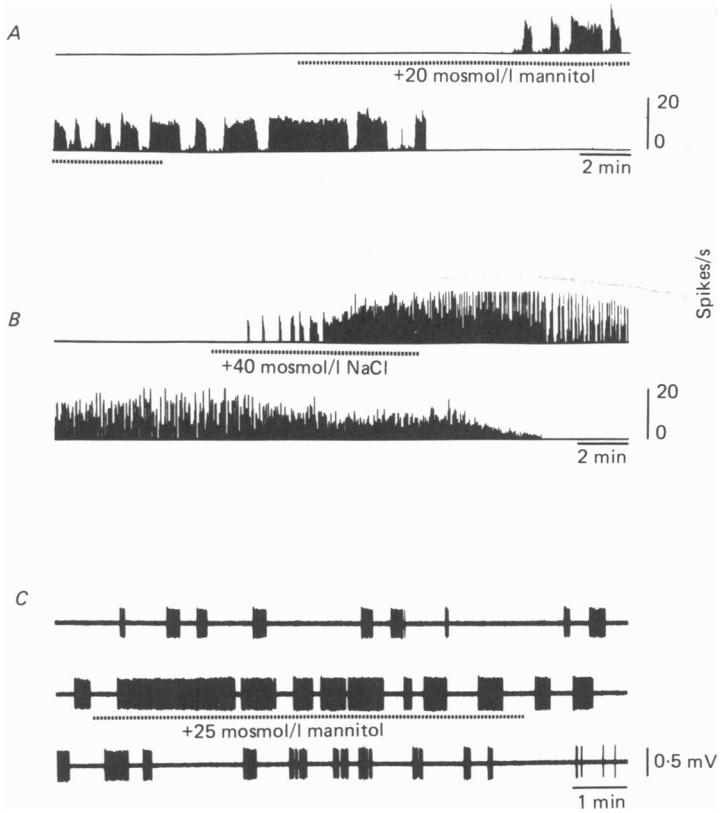

Fig. 4. *A*, *B*, rate-meter records from two silent supraoptic neurones illustrate the induction of phasic or bursting activity during perfusion with hyperosmotic media. In *A*, a +20 mosmol/l change in osmotic pressure prompts the appearance of phasic activity only. In *B*, the initial response to a larger (+40 mosmol/l) increase in osmotic pressure is characterized by phasic activity of increasing duration, proceeding through a period of continuous and bursting activity that persists for several minutes after the stimulus. *C*, continuous oscilloscope recording from another phasically active supraoptic neurone illustrates a reversible increase in burst length and a reduction in interburst interval during the exposure to hyperosmotic media.

Continuous firing. During osmotic stimulation, four of five initially silent neurones (Fig. 2*A*, upper trace) were induced to fire continuously, achieving an average rate of 9·3 Hz (range 2·4–20·2). Eight of nine cells with initial irregular or continuous activity (Fig. 2*A*, lower trace) also responded with a progressive increase of activity; their mean firing rates rose from 3·6 Hz (range 0·3–13·8) to 15·2 Hz (range 2·4–24·5).

The time course to peak and the recovery of the mean firing frequency of six neurones in this group are illustrated in Fig. 3*A*.

Phasic firing. We included in this group neurones which at some point during the recording session displayed phasic activity even though they were initially encountered as silent or continuously active cells.

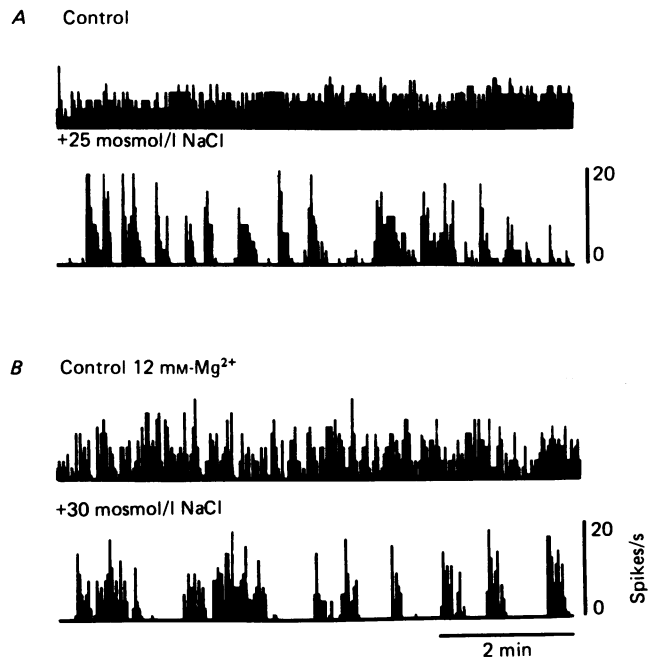

Fig. 5. *A*, excerpts from rate-meter records of a supraoptic neurone that demonstrated continuous spontaneous activity during perfusion with control media (upper trace) and phasic or bursting activity in hyperosmotic media (lower trace). *B*, records from another supraoptic neurone illustrate similar features obtained with media containing 12 mM-Mg^{2+}.

All of three initially silent neurones in this category responded to osmotic stimulation with immediate phasic firing (Fig. 4*A* and *B*). In each succeeding burst there was a trend towards prolongation of the burst duration and/or a reduction in the interburst interval (Fig 4*A*). Exposure to media with the highest osmotic pressures led to a replacement of this initial response with a secondary high-frequency bursting pattern outlasting the stimulus by as much as 30 min (Fig. 4*B*).

Two cells initially encountered with continuous activity were observed to change to phasic activity during exposure to hyperosmotic media (Fig. 5*A*). Six other neurones were initially encountered in the phasic mode of firing (Fig. 4*C*). In all of the latter, osmostimulation was associated with a combination of increased burst length, increased intraburst firing rate and decreased interburst interval (Figs. 4*C* and 6).

Activity patterns in 12 mM-Mg^{2+}

In order to ascertain the possible contribution of synaptic influences on the response to elevated osmotic pressure, we examined twenty-one additional supraoptic neurones using perfusion media containing 12 mM-Mg^{2+}, shown to be sufficient to

block synaptic transmission in this preparation (cf. Fig. 1). Although spontaneous activity was reduced in high-Mg^{2+}-containing solutions, cells with either continuous or phasic activities were present under control conditions. Furthermore, the majority of neurones tested were still responsive to sucrose (one of three cells), mannitol (five of eight cells) and NaCl (eight of ten cells) mediated increases in osmotic pressure ranging from 20 to 40 mosmol/l with patterns of response that were strikingly similar to those described in the preceding section.

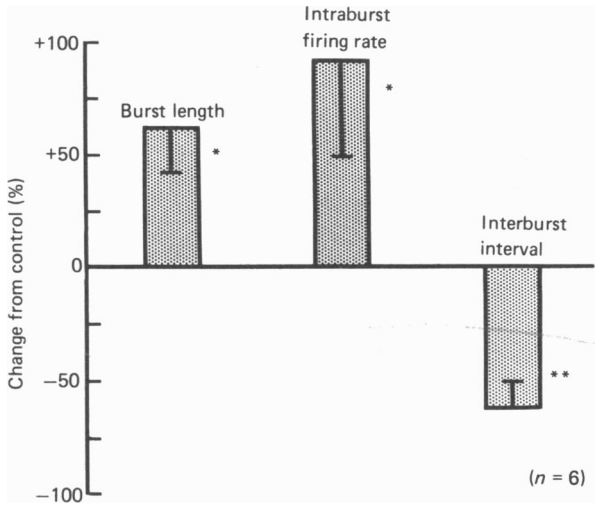

Fig. 6. A summary histogram obtained from seven phasically active cells indicates the mean (±S.E. of the mean) change in their burst length, intraburst firing frequency and interburst interval recorded during exposure to hyperosmotic media. (*$P < 0.05$; **$P < 0.005$, Student's t test.)

Continuous firing. During an osmotic stimulus, seven of ten silent neurones were induced to fire at a mean peak rate of 4·9 Hz (range 1·8–11·3) and four of eight cells with continuous activity were seen to increase their mean firing rate from 3·2 Hz (range 0·2–6·5) to 8·7 Hz (range 4·0–12·2). Fig. 2*B* illustrates typical responses while Fig. 3*B* represents the averaged response of all eleven neurones.

Phasic firing. Three cells observed to fire phasically in synaptic isolation were also tested with osmotic stimulation. One of these cells displayed an initial continuous irregular pattern but assumed a clear phasic activity in response to osmotic stimulation (Fig. 5*B*). The other two demonstrated a pattern similar to that shown in Fig. 4*C*, with increased burst duration and increased intraburst firing rates.

Osmosensitivity of other hypothalamic neurones

Previous studies *in vivo* indicate a sensitivity among other hypothalamic neurones to systemic osmotic stimuli (cf. Hayward, 1977 for review; Leng, 1982). In order to test the osmosensitivity of presumed non-neurosecretory cells, and to assess the specificity of the osmotic responses recorded from supraoptic neurones in the perfused explants, we monitored the extracellular activity of six neurones in the adjacent lateral hypothalamic area and thirteen cells in the medial hypothalamus during

exposure to +20 to +40 mosmol/l (Na^+) changes in osmotic pressure. With twelve cells, the perfusion media also contained 12 mM-Mg^{2+}. Although supraoptic neurones were consistently responsive in these preparations, none of these other hypothalamic cells demonstrated any significant change in firing during the osmotic stimulus.

DISCUSSION

A recent innovation in experimentation *in vitro* on mammalian central neurones is the use of intravascular perfusion to improve tissue viability during the assessment of cellular properties and interactions of neuronal networks in explants or whole mounts of brain tissue (Llinas, Yarom & Sugimori, 1981; Walton & Llinas, 1982; Bourque & Renaud, 1983*a*, *b*). This approach to electrophysiology of hypothalamic neurosecretory neurones offers certain advantages over hypothalamic slice (Hatton, Doran, Salm & Tweedle, 1980) and cultured explant (Armstrong & Sladek, 1982) preparations. First, antidromic and orthodromic responses can be elicited from readily identifiable regions. Secondly, the dense capillary network surrounding supraoptic neurones (Ambach & Palkovits, 1979) provides for extensive and intimate contacts between neuronal processes and capillary endothelium, an interface that must be viewed as important for these neurones to sense changes in plasma osmotic pressure. Thus, delivery by the intravascular route of solutions with increased osmotic pressure can be expected to provide an osmotic stimulus to supraoptic neurones that more closely resembles the condition *in vivo* of an increase in plasma osmotic pressure, and consequently to yield 'physiological' responses. Thirdly, the composition of the perfusion media can be easily altered during studies on the pharmacology of neurosecretory neurones.

The spontaneous and induced activity patterns of supraoptic neurones in perfused explants bear a striking similarity to those observed during recordings *in vivo* (cf. Poulain & Wakerley, 1982). Of particular note is the presence of phasic or bursting activity, deemed to be a characteristic of activated vasopressinergic neurones. There is considerable interest as to whether the mechanisms underlying this form of activity are endogenous to a subpopulation of neurosecretory neurones, or merely reflect the influence of activity in various afferent pathways in these cells. Although several studies *in vivo* illustrate that such phasic firing can be induced or modified by osmotic stimuli (Brimble & Dyball, 1977; Leng, 1980) and impulses arising in recurrent (Dreifuss, Tribollet, Baertschi & Lincoln, 1976; Leng, 1981) or various afferent pathways (Dreifuss, Harris & Tribollet, 1976; Harris, 1979; Poulain, Ellendorff & Vincent, 1980; Renaud, Arnauld, Cirino, Layton, Sgro & Siatitsas, 1981; Hamamura, Shibuki & Yagi, 1982; Thomson, 1982), *in vitro* recordings in the presence of sufficient Mg^{2+} to block synaptic transmission indicate that phasic or bursting activity continues to be observed not only spontaneously (cf. Hatton, 1982) but also in response to an osmotic stimulus (Fig. 5*B*). Hence, the mechanisms responsible for phasic activity would appear to be intrinsic to the neurosecretory cell. Recent intracellular observations indicate prominent hyperpolarizing and depolarizing after-potentials within supraoptic neurones; these can summate to form plateau potentials and cause sustained burst activity, suggesting an endogenous oscillator potential (Andrew & Dudek, 1982; Bourque & Renaud, 1983*a*; Mason, 1983). Extrinsic

influences, be they synaptic or osmotic, could then be viewed to modify rather than generate this endogenous rhythm.

Previous extracellular recordings from supraoptic neurones in slice preparations have not demonstrated consistent responses to osmotic stimuli (Haller & Wakerley, 1980; Noble & Wakerley, 1982). While we can offer no explanation for the discrepancy, the present *in vitro* observations indicate that the majority of supraoptic neurones are sensitive to the osmotic pressure of media in the forebrain vasculature, in close agreement with *in vivo* observations that the majority of supraoptic oxytocinergic and vasopressinergic neurones respond to systemic or local changes in osmotic pressure (Brimble & Dyball, 1977; Leng, 1980, 1982). Moreover, the osmotically induced patterns of activity recorded *in vitro* are very similar to those identified *in vivo*, including the appearance of enhanced firing frequency and the evolution of phasic activity patterns. *In vivo*, a 2 % increase in plasma osmotic pressure will induce a 2–3-fold increase in plasma vasopressin levels (Dunn *et al.* 1973). With *in vitro* studies, electrical events are detected with a 3–5 % increase in osmotic pressure of the surrounding media (cf. Mason, 1980). The higher *in vitro* thresholds noted in the present experiments may be due to lower temperatures of the preparations, and the isolation of supraoptic neurones from extrahypothalmic afferents that would normally augment their osmotic response.

Since supraoptic neurones are sensitive to an elevation in osmotic pressure mediated by either Na^+, sucrose or mannitol, and since any of these can produce essentially similar response patterns, it would appear reasonable to view the supraoptic neurone as a true osmoreceptor, rather than a simple Na^+ receptor (cf. Andersson, 1978). The mechanism responsible for enhanced neuronal excitability during an osmotic stimulus involves a membrane depolarization associated with a reduction in K^+ conductance (Abe & Ogata, 1982). Osmosensitivity may be a general property of all supraoptic neurones; intracellular recordings (Mason, 1980) indicate the presence of subthreshold depolarizations during osmotic stimulation, events that would escape detection during extracellular recordings. Both presynaptic and post-synaptic elements appear to participate in the osmotic response (Mason, 1980); a reduction in the average rate of rise to peak and in the over-all amplitude of the frequency response of supraoptic neurones in synaptic isolation (Fig. 3*B*) may simply reflect the role of these presynaptic components which themselves may be osmosensitive and which may serve to modulate the sensitivity of osmosensitive neurones and their over-all amplitude of response.

The available data indicate that supraoptic neurones are almost certainly osmosensitive (cf. Leng, Mason & Dyer, 1982). In the intact organism, a rise in plasma osmotic pressure is likely to affect not only the neurosecretory cells but also a network of neurones which will interact with the neurosecretory cells to modulate their endogenous osmosensitivity and lead to enhanced activity and hormone release. Synaptic inputs from areas such as the AV3V and adjacent rostral hypothalamic regions could serve to 'fine tune' the neurosecretory neurone, and thus achieve a balanced response suitable to the requirements of vascular volume, salt balance, blood pressure and other parameters. Thus, as originally proposed by Jewell & Verney (1957) the anterior hypothalamus and supraoptic region may be viewed as an osmosensitive functional unit that can respond to an osmotic stimulus itself, but can also act in concert with other osmosensitive regions.

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